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Research Notes: Identification of male sterile soybean plants by pollen examination

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1) Identification of male sterile soybean plants by pollen examination.

The male sterile maintainer line, N69-2774, segregates 1:1 for fertility and sterility (Brim and Young, 1972). The fertile and sterile siblings are indistinguishable, except for pollen grain morphology, until the onset of maturity. For controlled hybridization the phenotypic similarity is an obstacle. To avoid contamination, male fertile plants of the maintainer must be removed from crossing blocks as quickly as possible. Pollen grains of the male sterile plants are typically larger than those of fertile siblings, appear to be fewer in number, do not germinate or disperse in a germination medium, but remain in clumps similar to the crescent shape of the anther (Brim and Young, 1971). We describe here a precise and rapid technique of pollen examination to distinguish between male fertile and male sterile plants in a segregating population.

Flowers collected from individual plants in the population are desiccated over calcium chloride prior to examination. One hour of desiccation is sufficient for anther dehiscence unless relative humidity is unusually high. Two or more flowers are usually collected from each plant to reduce the risk of choosing flowers which will not dehisce. Because pollen sterility of the male sterile plants is complete, a single flower is enough to classify the plant. It should be noted that at Raleigh, NC it is more difficult to obtain suitable flowers for classification from greenhouse-grown plants. No such difficulties have been experienced with field-grown plants.

A hanging-drop slide of the type available from most glassware supply houses is used in classification. A 30% sucrose solution constitutes the germination medium. A single drop of the medium is placed on a 22 X 22 mm cover slip. The flower is emasculated to expose the anthers and pollen is dispersed in the media by gently tapping the flower above the drop. The rim of the hanging-drop slide is lubricated with vaseline which serves as an adhesive. The slide is then placed over the drop on the cover slip and quickly inverted.

The pollen grains in the drop are examined under a binocular microscope at 10X magnification. Pollen tube formation of fertile pollen grains is usually apparent in 15-20 minutes at room temperature (75-80°F). Once the flowers are collected one individual can classify approximately 50 slides per hour.

Pollen grains from male sterile plants are readily distinguished in the hanging drop. Occasionally, shrunken, well-dispersed pollen grains which do not germinate are detected. These are usually from anthers which have dehisced the day prior to sampling and upon resampling prove to be from fertile plants.

This method provides a technique for removing fertile plants from the maintainer line in order to achieve controlled natural hybridization. Because the maintainer line has white flowers and grey pubescence, pollen donors carrying dominant markers can be used as parents in natural crosses thus avoiding the need to identify fertile plants in the maintainer. But where appropriate pollen donors are unavailable for the experimenters' purposes, then classification as outlined above is necessary.

References

Brim, C. A. and M. F. Young. 1971. Inheritance of a male-sterile character in soybeans. Crop Sci. 11: 564-567.

Brim, C. A. and M. F. Young. 1972. Registration of a male-sterile maintainer line (N69-2774) of soybeans. Crop Sci. 12: 339.

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1) Induction of sterility in soybeans with ethidium bromide.

Ethidium bromide (EB), an acridine compound which binds to nucleic acids, has been used as a mutagen in peanuts by Levy and Ashri (1975). Burton and Hanna (1976) induced cytoplasmic male sterile mutations in pearl millet with EB and suggested that EB as a cytoplasmic mutagen might aid in the development of a cytoplasmic male sterile nuclear restorer system in other crops. The following is a report on preliminary attempts to induce cytoplasmic male